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Evidence of unique and shared responses to major biotic and abiotic stresses in chickpea

Nitin L. Mantri[a,*], Rebecca Ford[b], Tristan E. Coram[c], Edwin C.K. Pang[a]

[a] RMIT University, School of Applied Sciences, Biotechnology and Environmental Biology, Building 223, Level 1, Plenty Road, Bundoora, 3083 Victoria, Australia
[b] Biomarka, Melbourne School of Land and Environment, The University of Melbourne, 3010 Victoria, Australia
[c] United States Department of Agriculture, Agricultural Research Service, Eastern Regional Small Grains Genotyping Laboratory and Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620, USA

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ABSTRACT

Microarrays have been used extensively for transcriptional profiling of plant responses to biotic and abiotic stresses. However, most studies focused on either biotic or abiotic stresses, making it difficult to construe the genes that that may be common to both biotic and abiotic-stress responses. Such information may help molecular breeders to develop cultivars with broad-spectrum resistances to these stresses. A 768-featured boutique microarray was employed to compare the genes expressed by chickpea in response to drought, cold, high salinity and the fungal pathogen *Ascochyta rabiei* and 46, 54, 266 and 51 differentially expressed transcripts were identified, respectively. The expression of common genes indicated crosstalk in the genetic pathways involved in responses to these stress conditions. The response of ICC 3996 to *A. rabiei* was more similar to that of high-salinity stress than to drought or cold stress conditions.

1. Introduction

Although valued for its high nutritional and protein composition (Ahmad et al., 2005), cultivation of chickpea is constrained by many biotic and abiotic stresses. These include *Ascochyta* blight (*Ascochyta rabiei*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *ciceri*), and pod borer (*Helicoverpa armigera*) as well as drought, heat, cold and salinity (Ryan, 1997). Efforts to breed for improved stress tolerances have involved germplasm screening and genetic mapping with the future view towards marker-assisted trait selection (Millan et al., 2006; Toker et al., 2007). However, cultivated chickpea contains narrow genetic variation (Udupa et al., 1993) and there is limited information on the presence of desirable traits in wide(r) germplasm sources. Together with the likely complex genetics governing their expressions, it is proposed that these constraints have resulted in the slow progress to develop elite varieties that are disease resistant and environment tolerant. Rather than targeting specific genes underpinning a trait of interest, breeding efforts have relied on selection of associated morphological expression such as higher root density as an adaptive response for drought tolerance. Since multiple genes govern stress tolerance quantitatively, screening for tolerance based on phenotype in a static environmental bioassay is somewhat unreliable (Foolad, 1999). Hence, identification, characterisation of expression and selection of a suite genes underpinning a particular stress tolerance under a particular environmental stimuli would enable far more effective breeding strategies.

Mechanisms of stress response and tolerance are governed by the transcriptional activation and repression of genes that are involved in stress perception to actual response or adaptation (Vinocur and Altman, 2005; Pieterse and Dicke, 2007). The expression and interaction of these genes is complex and diverse, and every gene involved forms part of a coordinated response network. The speed and coordination of expression of these genes is vital for plant survival.

Gene expression profiling using microarrays serves as an excellent platform to identify and compare genes expressed by plants in response to a multitude of stresses. Stress specific and shared pathways with crosstalk in gene expressions between abiotic-stress responses has previously been detected (Knight and Knight, 2001; Seki et al., 2003). Much of this research has been conducted with the model crop genomes, for which a wealth of knowledge exists. Treatment with cold, salt, osmoticum, wounding, jasmonic acid and pathogens on *Arabidopsis thaliana* identified a number of previously characterised transcription factor genes that changed significantly and commonly across stress stimuli, suggesting their multifunctional nature (Chen et al., 2002; Cheong et al., 2002). Recently,

* Corresponding author. Tel.: +61 3 9925 7140, fax: +61 3 9925 7110.
E-mail address: nitin.mantri@rmit.edu.au (N.L. Mantri).

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crosstalk between abiotic and biotic stress responses and points of convergence in stress signalling networks was reviewed and several transcription factors and kinases were proposed as key crosstalk controllers between stress signalling pathways (Fujita et al., 2006). The identification, characterisation of expression and selection of genes that are specific as well as those that are shared among responses to biotic and abiotic stresses will enable breeders to develop elite genotypes with broad-spectrum tolerance and adaptation.

However, the chickpea genome remains very much a black box with very little genomic information available. In a previous study, an *Ascochyta* blight resistance cDNA library was constructed from ICC 3996, a *desi* chickpea accession with high *A. rabiei* resistance (Nasir et al., 2000; Collard et al., 2001). Using 559 of the unique sequenced clones (Coram and Pang, 2005), 156 grasspea cDNAs (Skiba et al., 2005) and 41 lentil RGAs (Barkat Mustafa, personal communication), boutique array (PulseChip) was constructed. This was used to study the response to *A. rabiei* by Coram and Pang (2006) and to reveal putative genes/pathways associated with tolerance/susceptibility to abiotic stresses of drought, cold and high salinity (Mantri et al., 2007) in chickpea. However, since these studies were conducted in separate genotypes, common gene/pathway involvement among stress stimuli were not able to be accurately identified. Therefore, in the present study, ICC 3996 plants were challenged with drought, cold and high-salinity stresses to reveal the genes commonly expressed. These were subsequently compared with those previously reported to be involved in the *A. rabiei* response.

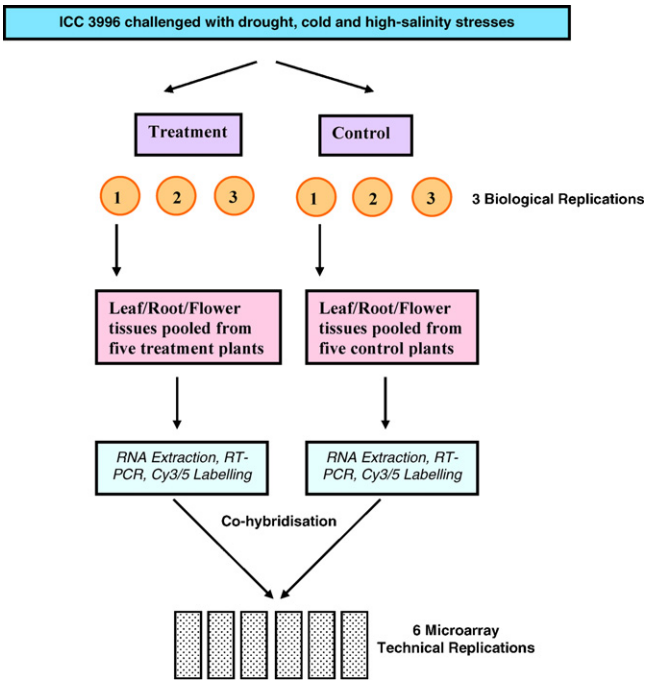

Fig. 1. Flow-chart showing the stress treatment procedure and tissue sample processing to generate gene expression profiles. The high-salinity stress treatment included two time-points (24 and 48 h) at which the tissues were harvested.

2. Methods

2.1. Experimental design

The experimental design of this study was carefully chosen to allow comparison of genes expressed by chickpea genotype ICC 3996 in response to biotic stress (*Ascochyta* blight) and abiotic stresses (drought, cold, and high salinity). The results from an earlier study on expression profiling of ICC 3996 in response to *A. rabiei* (Coram and Pang, 2006) were directly used. They documented the response of 14-day-old ICC 3996 plants to *A. rabiei* at 6, 12, 24, 48 and 72 h post-inoculation (hpi). In this study, ICC 3996 plants were cultivated and challenged with drought, cold, and high-salinity stresses as described by Mantri et al. (2007). Briefly, five treatment and five control plants were grown for each abiotic-stress condition. For drought and cold stress study, plants were cultivated in sterile potting mix (one plant per 15 cm pot) in a glasshouse at 15–25 °C. Drought or cold stress was imposed on treatment plants 2 weeks after flowering whilst the control plants were grown normally. The leaf, root, and/or flower tissues were harvested for stress response analysis as described (Mantri et al., 2007). For high-salinity stress study, five treatment and five control plants were cultivated in a hydroponic system using 50 L plastic crates. High-salinity stress was imposed on 18-day-old treatment plants by adding 150 mM sodium chloride (NaCl) (pH 6.5) to their growth media whilst the control plants were grown normally. The shoot and root tissues were collected 12 and 24 h post-treatment (hpt) for gene expression analysis. All stress treatment experiments were performed in three biological replicates (Fig. 1). The 768-feature boutique 'Pulse Chip' microarray consisting of 559 chickpea cDNAs, 156 grasspea cDNAs, 41 lentil resistance gene analogs (RGAs) and 12 controls used by Coram and Pang (2006) was employed for hybridization to allow effective comparison. Moreover, the same hybridization, scanning, and differentially expressed (DE) gene selection protocol as Coram and Pang (2006) was used. Data transformations consisted of a local background correction, omitting flagged spots, normalization by applying the LOWESS algorithm, creating a Cy5/Cy3 mean signal ratio, $\log_2$ conversion, and combining replicates. To identify DE genes, expression ratio results were filtered to eliminate genes whose 95% confidence interval for mean fold change (FC) did not extend to two-fold up or down. These cut-offs translated into induced transcripts having a $\log_2$ ratio ≥ 1.0 and repressed transcripts a ratio of ≤ -1.0. This was followed by a Student's *t*-test and False Discovery Rate (FDR) multiple testing correction to retain only genes in which expression changes *v.* unstressed control were significant at $P < 0.05$. The microarray results for abiotic-stress response of ICC 3996 were validated by quantitative real time polymerase chain reaction (qRT-PCR) as described by Mantri et al. (2007).

3. Results and discussion

3.1. Experimental design and analysis

The ICC 3996 plants were grown, challenged with drought, cold, and high-salinity stresses, and tissues were collected as previously described (Mantri et al., 2007). A standardised system of plant growth, stress treatment and replication was used in order to minimise experimental variability and ensure accurate measurements of changes in mRNA abundance (Mantri et al., 2007). The experiments were conducted in a reference design where respective tissues from unstressed plants served as controls. A stringent two-fold cut-off combined with Student's *t*-test ($P < 0.05$) ranking and FDR multiple testing correction selection was used to select transcripts differentially expressed (DE) between stressed and unstressed/control plants. All Minimum Information About a Microarray Experiment (MIAME) guidelines were followed and the datasets were deposited into the Gene Expression Omnibus, National Center for Biotechnology Information (series no. GSE8554). The microarray observations were validated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Eight transcripts with different expression values and representing different stresses, tissue-types and/or time-points were selected for

Table 1
Expression ratios of selected transcripts assessed by microarray and qRT-PCR.

Treatment/tissue-type/time-point	GenBank accession	Category	Putative function	Array	qRT-PCR
Drought leaves	DY475477	Cellular metabolism	Asparagine synthetase (glutamine hydrolysing) (EC 6.3.5.4)-induced by the dark	1.27	2.65
Drought flowers	EB085047	Protein synthesis	18S rRNA	−3.29	−4.71
Cold leaves	DY475403	Cellular metabolism	Carbonic anhydrase like protein (EC 4.2.1.1) - reversible hydration of carbon dioxide	−1.15	−2.77
Cold flowers	DY475275	Unknown	Unknown	−2.45	−3.53
Salt shoot 24 hpt	DY475260	Unknown	Unknown	−1.16	−2.41
Salt root 24 hpt	DY475384	Cellular communication and signalling	Serine/Threonine-like protein kinase	−3.44	−3.83
Salt shoot 48 hpt	DY475154	Transcription	Chloroplast 4.5S/5S/16S/23S mRNA	−1.02	−2.39
Salt root 48 hpt	DY475408	Cellular metabolism	Xylosidase	2.51	3.62

Array values indicate mean $\log_2$ fold change (FC) ratio relative to untreated controls and qRT-PCR values indicate $\log_2$ ratios of $2^{(\Delta C_t \text{control}/\Delta C_t \text{treatment})}$. A set of DE genes with different expression values from different stress treatments, tissue-types and/or time-points were chosen for qRT-PCR confirmation.

qRT-PCR validation. The comparison of expression values between the two methods revealed similar expression kinetics for all the genes tested indicating reliability of microarray data (Table 1). However, the fold change values obtained through qRT-PCR were generally more exaggerated than the corresponding microarray values. Similar observations were reported in other microarray studies (Dowd et al., 2004; Lopez et al., 2005; Mantri et al., 2007).

3.2. Identification of abiotic-stress response in ICC 3996

The transcripts with an altered up- or down-regulated transcription level were observed following each of the stress responses among the tissue-types assessed. The breakdown by stress for the 756 probes (representing ESTs and RGAs) identified a two-fold or greater change in expression in a total of 317 ESTs. The number of DE transcripts affected in response to high salinity (266) was higher than those affected in response to drought (46) and cold (54) stresses. A similar observation was made in our earlier study where stress tolerant and susceptible genotypes were screened against drought, cold and high-salinity stresses (Mantri et al., 2007). The list of transcripts DE in response to drought, cold and high-salinity stresses is presented in Supplementary Tables 1–3. The possible involvement of some important transcripts in adaptation to these abiotic stresses was previously discussed in detail (Mantri et al., 2007). Here we briefly highlight these transcripts followed by discussion on interesting transcripts DE in response to biotic and abiotic stresses.

3.3. Transcripts similarly expressed to earlier study

The following transcripts found to be expressed in ICC 3996 in the current study were also found to be DE in an earlier study focused on abiotic-stress responses in chickpea. The function and putative role/involvement of these transcripts in adaptation to abiotic stresses has been discussed (Mantri et al., 2007). These included transcripts associated with senescence like S-adenosylmethionine synthetase (DY475190) that was suppressed under drought and high-salinity stresses. Further, transcripts associated with energy metabolism/photosynthesis (EB085050, DY396330, DY475518, DY475316, DY475116, DY475069, DY475454, DY475345, DY475142, and DY475305) were repressed in the drought, cold, or high-salinity stressed plants. Among the transcripts related to transport, aquaporins (DY475174 and DY396334) were repressed whilst the transcript for a DNA-J like protein involved in intra-cellular protein transport (DY475488) was induced in response to cold and high-salinity stresses. Transcripts DE and associated with pathogen defence included pathogenesis-related protein (DY396305), auxin-repressed protein (DY396359), caffeoyl-CoA O-methyltransferase (CV793595), glycine-rich cell wall protein GRP 1.8 (DY396342), pathogenesis-related protein 4A (DY396281 and CV793597) were also induced and/or repressed in response to drought, cold, and high-salinity stresses. The expression of similar transcripts in this and previous study supports the hypothesis of their involvement in abiotic-stress response in chickpea.

3.4. Comparison of biotic and abiotic-stress responses of ICC 3996

DNA microarrays have been considered to be an excellent platform for comparison of genes expressed by plants under biotic and abiotic stresses. Stress specific and shared pathways have been unveiled by such comparisons allowing the detection of points of cross-talk between these stress responses (Chen et al., 2002; Cheong et al., 2002). The 'Pulse Chip' array was constructed in association with Mr. Tristan Coram, who used it to generate expression profile of chickpeas in response to *Ascochyta* blight pathogen (Coram and Pang, 2006). ICC 3996, which is *Ascochyta* blight resistant genotype, was one of the genotypes studied by Coram and Pang (2006). Hence, the comparison of genes DE by ICC 3996 in response to abiotic stresses from this study and biotic stress from Coram and Pang (2006) was considered, to detect genes commonly expressed under these stresses.

The comparison of genes DE by ICC 3996 in response to drought, cold, high-salinity and *A. rabiei* stresses in different tissue-types and/or time-points is presented in Fig. 2. Globally, 46, 54, 266, and 51 transcripts were DE in at least one tissue-type or time-point in response to drought, cold, high salinity, and *A. rabiei*, respectively. The numbers indicated in the blocks are transcripts exclusively DE for that particular combination and are not repeated in other subset/superset combinations. Thirty transcripts were uniquely DE in response to *A. rabiei*, whilst no transcript was commonly DE across all the four stresses assessed.

Twenty-one transcripts were commonly DE among the *A. rabiei* and one or more of the abiotic stresses. The number of transcripts that were commonly DE between the *A. rabiei* and high-salinity stresses (16) was about twice and thrice those commonly DE between *A. rabiei* and cold stresses, and *A. rabiei* and drought stresses, respectively (Table 2). This may be because *Ascochyta* blight and high-salinity stress response involve similar pathways. However, it should be noted that these stresses were both studied at seedling stage, whereas drought and cold stresses were studied at flowering stage. Therefore, the DE genes identified may also be related to tissue growth stage. Moreover, for both, *Ascochyta* blight and high-salinity stresses, the shoot tissues (stem and leaves pooled

Table 2
The ESTs commonly DE by ICC 3996 in response to drought, cold, high-salinity, and *Ascochyta* blight stresses.

GenBank accession	Category	Putative function	Treatment/tissue-type/time-point[a]	Log_2 ratio	*P*-Value
Drought and Ascochyta blight					
None					
Cold and Ascochyta blight					
DY475157	Unknown	Unknown	CL	−2.79	4.43E−12
			AS 72	0.75	0.257669
CV793591	Defence	S1-3 protein homolog induced by CMV infection in cowpea	CF	−2.06	0.003089
			AS 48	−0.54	0.377882
DY475478	Cellular communication/Signal transduction	Hypothetical transmembrane protein	CF	−1.08	4.3E−21
			AS 48	−0.88	0.159919
			AS 72	−1.02	0.315523
High salinity and Ascochyta blight					
DY475532	Unknown	Unknown	SS 24	−3.91	0.002386
			AS 48	1.00	0.194921
DY396305	Defence	Pathogenesis-related protein	SS 48	−2.47	7.41E−10
			AS 48	0.73	0.180017
DY475150	Protein synthesis/fate	18S nuclear rRNA	SR 24	1.27	3.74E−13
			SR 48	1.01	1.22E−13
			AS 12	0.58	0.289257
DY475220	Cell rescue/death/ageing	Wound-induced protein	SS 24	−2.33	0.006977
			SS 48	−2.74	0.000113
			AS 72	−0.75	0.253955
DY475305	Energy	Thylakoid protein	SS 48	−1.92	0.000822
			AS 48	−0.58	0.375538
CV793597	Defence	Pathogenesis-related protein 4A	SS 48	−1.07	0.007476
			AS 24	0.97	0.247602
CV793606	Defence	Homologous to SNAKIN2 antimicrobial peptide induced by pathogen infection	SS 24	1.80	0.000553
			SS 48	2.34	5.49E−10
			AS 24	0.86	0.264627
DY475357	Cell cycle and DNA processing	RNA/ssDNA binding protein	SS 24	−1.35	0.000958
			SS 48	−1.14	0.00142
			AS 12	−0.86	0.050468
			AS 48	−1.23	0.18067
DY475365	Unknown	Unknown	SS 24	−1.26	8.22E−06
			SS 48	−1.00	4.78E−07
			AS 48	0.89	0.189309
DY475384	Cellular communication/Signal transduction	similar to serine/threonine protein kinase	SR 24	−3.45	4.37E−40
			AS 24	−0.77	0.345518
			AS 72	−0.63	0.233938
Drought, cold and Ascochyta blight					
DY475172	Cell rescue/death/ageing	Phosphate-induced protein	DL	−1.13	3.51E−11
			CL	4.12	0.000791
			AS 48	−0.86	0.132234
DY475181	Cellular metabolism	Apocytochrome F	DL	−1.82	0.003616
			CL	−1.11	5.56E−11
			AS 24	−1.14	0.095093
Drought, high salinity and Ascochyta blight					
DY475116	Energy	Photosystem II reaction centre I protein	DL	−2.43	6.34E−13
			SS 24	−2.41	8.2E−15
			AS 48	−1.08	0.183149
DY475190	Cell rescue/death/ageing	S-adenosylmethionine synthetase (EC 2.5.1.6) differentially expressed after stress	DL	−2.64	1.39E−08
			SS 24	−1.48	1.53E−07
			AS 24	−0.90	0.296914
DY475302	Cellular metabolism	4-alpha-Glucanotransferase (EC 2.4.1.25)	AS 72	−1.05	0.079218
			DL	−1.97	3.89E−14
			SR 48	1.04	1.54E−09
			SS 24	−4.28	5.14E−05
			SS 48	−2.24	1.13E−05
			AS 72	−0.82	0.141383
Cold, high salinity and Ascochyta blight					
DY475076	Cell rescue/death/ageing	Phosphate-induced protein	CF	1.79	0.000634
			CL	3.02	1.52E−16
			SS 24	−3.45	4.63E−09
			AS 12	−1.23	0.271166
DY475550	Cellular communication/signal transduction	WD-repeat protein (trp-asp domains) involved in protein–protein interactions including signal transduction, transcription regulation and apoptosis	CL	−1.02	0.002221
			SS 24	−4.16	9.62E−07
			SS 48	−1.57	0.000214
			AS 48	−0.78	0.098255
DY475403	Cellular metabolism	Carbonic anhydrase like protein (EC 4.2.1.1)-reversible hydration of carbon dioxide	CL	−1.15	0.000244

Table 2 (*Continued*)

GenBank accession	Category	Putative function	Treatment/tissue-type/time-point[a]	Log_2 ratio	*P*-Value
			SS 24	−2.78	3.27E−06
			AS 72	−0.66	0.39002
Drought, cold, high salinity and Ascochyta blight					
None					

[a] DL: drought leaves; DF: drought flowers; CL: cold leaves; CF: cold flowers; SS 24: high-salinity shoots 24 hpt; SS 48: high-salinity shoots 48 hpt; SR 24: high-salinity roots 24 hpt; SR 48: high-salinity roots 48 hpt; AS 12: *Ascochyta* blight shoots 12 hpt; AS 24: *Ascochyta* blight shoots 24 hpt; AS 48: *Ascochyta* blight shoots 48 hpt; AS 72: *Ascochyta* blight shoots 72 hpt.

together) were studied, whilst for drought and cold stresses, only leaf tissues were studied.

The transcripts commonly DE under various combinations of biotic and abiotic stresses were either similarly or differently expressed (induced under one stress whilst being repressed under other or induced/repressed under both stresses). The complete list of these transcripts is available in Table 2. Here we limit this discussion on a few interesting transcripts that were similarly/differently expressed under biotic and abiotic stresses.

The transcript associated with wound-induced protein (DY475220) involved in cell rescue/death/ageing was repressed in shoots under *A. rabiei* stress at 72 h post-inoculation (hpi) and high-salinity stress at 24 and 48 h post-treatment (hpt). Salicylic acid is critical in signalling plant defense against pathogen attack (Klessig et al., 2000). Salicylic acid is also a known inhibitor of wound-induced genes (Li et al., 1992). Therefore, it may be possible that ICC 3996, an *A. rabiei* resistant genotype, employed a salicylic acid signaling pathway to battle the pathogen which was suppressing the wound-induced protein. Moreover, the adaptation to high-salinity stress may also involve a salicylic acid signalled pathway.

Interestingly, phosphate-induced protein transcript (DY475172) was repressed in the leaves of drought stressed plants and shoots of *Ascochyta* blight stressed plants at 48 hpi, while it was 16-fold induced in the leaves of cold-stressed plants. Another phosphate-induced protein transcript (DY475076) was repressed in the shoots of high-salinity and *A. rabiei* stressed plants (24 hpt and 12 hpi, respectively) whilst being three- to eight-fold induced in the flowers and leaves of cold-stressed plants. Phosphate-induced proteins are related to senescence and therefore its repression in drought, high-salinity and *Ascochyta* blight stressed plants may signify effort being made by the plants to delay death. In fact, delay of senescence has been considered as one of the mechanisms of tolerance in other crops (Borrell et al., 2000; Yan et al., 2004). However this protein may have a different role in response to cold stress. Mitogen activated protein kinases (MAPKs) play a central role in stress signalling, and are proposed to be involved in cold acclimation of plants (Chinnusamy et al., 2006). Evidence for the activation of MAPKs by phosphate-induced cell-cycle entry of tobacco cells was previously reported (Wilson et al., 1998). Hence, the phosphate-induced proteins may be involved in activation of the MAPK signalling cascade, leading to cold acclimation of ICC 3996 plants.

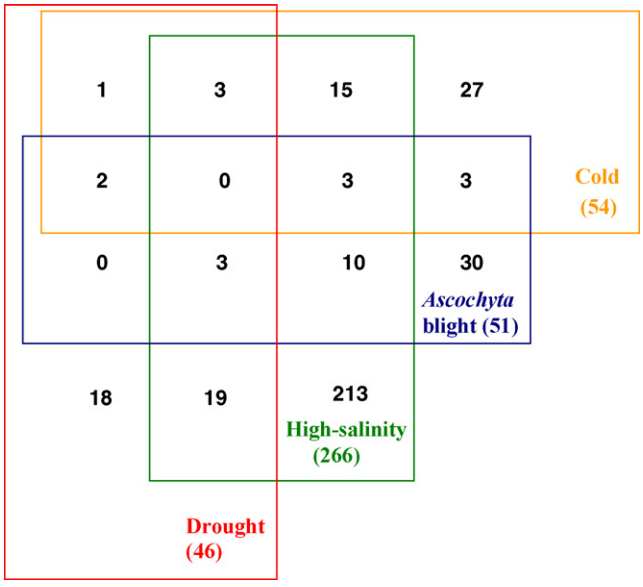

Fig. 2. Venn diagram comparing the transcripts that were DE by ICC 3996 in response to drought, cold, high-salinity and *Ascochyta* blight stresses.

The assumption that plants try to delay senescence whilst making an effort to adapt to stress is corroborated by repression of S-adenosylmethionine synthetase (DY475190) transcript. DY475190 related to senescence was repressed in the leaves/shoots of drought, high-salinity (24 hpt), and *Ascochyta* blight (24 and 72 hpi) stressed plants. It is interesting to note that the same gene was suppressed under biotic/abiotic stresses suggesting involvement of similar pathways for stress adaptation.

Among the genes related to plant defence, SNAKIN2 antimicrobial peptide (CV793606) induced by pathogen infection was three- to four-fold induced in the shoots of high-salinity stressed plants (24 and 48 hpt), and about two-fold induced in shoots of *Ascochyta* blight stressed plants (24 hpi). The genes related to pathogen defence have been previously reported to be induced under salt stress (Munns, 2005) but their role in stress adaptation still remains unknown. Also, the photosystem II reaction centre I protein (DY475116) transcript related to energy metabolism was repressed under drought, high-salinity and *Ascochyta* blight stresses. The genes involved in photosynthesis are known to be repressed in shoots following the treatment of plants with NaCl (salt stress), PEG (osmotic stress) or ABA. This response is consistent with the closure of stomata in response to high ABA or osmotic stress, inhibition of CO_2 fixation and reduced need for energy capture by photosynthetic ETC (Buchanan et al., 2005).

Further, it was observed that although plant growth was affected under high-salinity and *A. rabiei* stresses, more proteins were being synthesized; perhaps to combat stress. The RNA/ssDNA binding protein transcript (DY475357) related to cell cycle/DNA processing was suppressed whilst 18S nuclear rRNA transcript (DY475150) related to protein synthesis was induced under high-salinity and *A. rabiei* stresses.

Among the genes related to cellular metabolism, apocytochrome *f* transcript (DY475181) was repressed in the leaves/shoots of drought, cold and *A. rabiei* stressed plants. The role of apocytochrome *f* in stress response is not well understood. However, the release of cytochrome *c* and mitochondria have been shown to be involved in programmed cell death (PCD) induced by acetic acid in yeast (Ludovico et al., 2002). Thus, it may be possible that repression of apocytochrome *f* may help ICC 3996 plants to prevent PCD under these stress conditions.

Also, the 4-alpha-glucanotransferase transcript (DY475302) was 2–16-fold repressed in the leaves/shoots of drought, high-salinity (24 and 48 hpt), and *Ascochyta* blight (72 hpi) stressed plants, whilst being induced in the roots of high-salinity stressed plants (48 hpt). alpha-Glucanotransferase (AGT) is associated with the breakdown of starch into sucrose (Zeeman et al., 2004). Another transcript associated with starch degradation, alpha amylase (DY396337) (Asatsuma et al., 2005) was repressed in shoots in response to high salinity (24 and 48 hpt). Starch and sucrose molecules were reported to serve as reciprocal fluxes to each other (Zeeman et al., 2004). Sucrose is a known osmolyte that accumulates in salt-stressed plants (Munns, 2005). The repression of starch degradation pathways may mean that sucrose was being produced by alternative pathway or some other osmolyte may have been deployed to maintain cell-turgor under salt-stress. One observation related to this assumption is induction of the transcript associated with sucrose synthase (DY475105) in roots at 24 and 48 hpt. Sucrose synthase (SS) catalyses the reversible reaction of sucrose synthesis from glucose and fructose. Another observation was >four-fold induction of the transcript related to glutamate dehydrogenase (DY475308) in roots at 24 and 48 hpt. A study on salinity tolerance in wheat revealed that under high-salinity conditions, glutamate dehydrogenase is preferentially employed for production of proline. Proline is an osmolyte and transgenic plants engineered to over-accumulate proline showed enhanced salt tolerance (Zhu et al., 1998; Hong et al., 2000).

Further, carbonic anhydrase transcript (DY475403) involved in reversible hydration of carbon dioxide was repressed in leaves/shoots under cold, high-salinity (24 hpt) and *Ascochyta* blight (72 hpi) stresses. Carbonic anhydrase is involved in diverse biological processes including pH regulation, ion exchange, CO_2 transfer, respiration and photosynthetic CO_2 fixation (Tiwari et al., 2005). Thus, its repression under these different stresses may have varied roles and needs further investigation. For example, its repression under cold stress may be due to disruption of respiration/photosynthesis whilst repression under high-salinity stress may be to regain ionic balance.

Interestingly, two regulatory genes were also similarly expressed under the biotic and abiotic-stress conditions. The serine/threonine protein kinase transcript (DY475384) was repressed under high-salinity (24 hpt) and *Ascochyta* blight (24 and 72 hpi) stresses. Plant serine/threonine kinases are a huge family and those reported from *Arabidopsis thaliana* have been divided into around a dozen major groups based on their sequence relationships (Hardie, 1999). The protein kinases are mostly induced in plants under biotic and abiotic stresses and regulate the expression of downstream stress-responsive genes. However, some protein kinases like lucine rich receptor-like protein kinase are repressed by environmental stresses like light (Hardie, 1999). Moreover, a serine/threonine protein kinase, *Sac3*, has been shown to both positively and negatively regulate the response of cell to stress conditions in *Chlamydomonas* (Davies et al., 1999). Therefore, it may be possible that DY475384 was negatively regulating responses of ICC 3996 to high-salinity and *Ascochyta* blight stresses.

Further, the WD-repeat protein (trp-asp domains) transcript (DY475550) involved in protein–protein interactions including signal transduction, transcription regulation and apoptosis was 2–16-fold repressed in leaves/shoots of cold, high-salinity (24 and 48 hpt) and *Ascochyta* blight (48 hpi) stresses. WD-repeat (WDR) proteins are essentially involved in different cellular and organismal processes, including cell division and cytokinesis, apoptosis, light signalling, flowering, floral development, and meristem organisation (van Nocker and Ludwig, 2003). Its repression in the leaves/shoots under these abiotic and biotic stresses may suggest that it negatively regulates the expression of downstream genes required for stress adaptation/tolerance for these stresses.

4. Conclusions and perspectives

This study provided several insights on the genes and pathways unique and shared among responses to biotic and abiotic stresses in chickpea. Overall, 46, 54, 266 and 51 transcripts were identified as DE under drought, cold, high-salinity, and *A. rabiei* stresses, respectively. Several shared responses were observed indicating potential crosstalk between responses to these stress conditions. The response of ICC 3996 to *Ascochyta* blight was more similar to high-salinity stress than to drought or cold stress conditions. Interesting observations were made when the transcripts commonly expressed under biotic and abiotic stresses were analysed in context of their biological role in plants. However, it should be noted that the 'PulseChip' array used was representative of a limited number of genes from the chickpea genome. For more in-depth analysis, a larger number of ESTs and preferentially the complete chickpea genome are required.

Although this study provided several insights on the genes and pathways involved in biotic and abiotic-stress tolerance/adaptation in chickpea, definitive evidence is still lacking. This is because microarray studies merely provide "guilt by association" inferences. Changes in mRNA accumulation may not correlate with protein/enzyme activity levels (Gygi et al., 1999). However, sufficient information was obtained in this study to formulate hypotheses concerning biotic and abiotic-stress responses mechanisms in chickpea, which can be tested in future studies. The first future logical step would be to convert the candidate ESTs into molecular markers and map these onto the integrated chickpea genomic linkage map. Subsequently, the quantitative trait loci (QTLs) for drought, cold, high-salinity and *Ascochyta* blight stresses should be identified to see if any of the candidate ESTs co-localise with the respective QTLs. The co-localisation of the candidate ESTs with the respective QTLs may bolster their case of being possibly associated with stress adaptation/tolerance. Secondly, more in-depth expression studies involving the use of additional genotypes and more time-points supplemented with physiological observations during stress imposition may possibly provide a better insight into the role/involvement of the proposed genes/mechanisms in biotic/abiotic-stress responses in chickpea. Finally, important candidates can be short listed and their proof-of-function established using knockouts/TILLING-mutants/overexpressing-transgenics.

Nonetheless, the annotation of transcripts DE between stressed and unstressed plants strongly suggests that these putative genes have a role in biotic/abiotic-stress adaptation/tolerance in chickpea. Gene expression profiles provide a starting point for detailed studies on candidate genes to help prioritise the cumbersome task of using reverse genetics to assign gene functions (Kreps et al., 2002). The identification of genes involved in adaptation/tolerance to these stresses and their required timing of expression shall greatly aid development of elite chickpea cultivars through molecular breeding or genetic manipulation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2010.05.003.

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